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Results of Surveillance for *Vibrio cholerae* in Cuban Waters

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SHORT PAPERS AND NOTES

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RESULTS OF SURVEILLANCE FOR *VIBRIO CHOLERAE* IN CUBAN WATERS.—Cholera is a disease caused by bacteria of the *Vibrio cholerae* species that has affected world populations during many earlier episodes. In general, cholera is most likely in countries with deficient excreta disposal, deficient water supply, limited food hygiene, or poor application of appropriate sanitary measures. Beginning in the 18th century, John Snow and Robert Koch investigated the risk factors associated with cholera and others etiological agents that cause diarrhea. Koch first described the isolation of *V. cholerae* in water used for drinking and cooking. He emphasized the importance of diverse environmental reservoirs and control measures to avoid the transmission of diarrheal diseases. Both investigators recommended the consumption of secure water and initiation of programs of population education, to avoid the risks of consuming raw food and untreated water.

Snow described the incidence of cholera south of London during the summer of 1854 and established an association between the cases of cholera and a water supply from the Thames River. He demonstrated that those homes with deficient water supply had 315 deaths per 10,000 inhabitants, whereas in homes with better water quality, the rate was only 37 cases per 10,000 inhabitants.

Since this earlier research, in which the presence of cholera in water was considered the main way for transmission of the pathogen, later studies have considered additional water-related reservoirs. The importance of monitoring water and aquatic ecosystems has increased because it has been demonstrated that this pathogen can also be detected in aquatic plants, algae, plankton, and seafood.

After the seventh pandemic of cholera in Peru in 1991 and its propagation into parts of Latin America, introduction into the Caribbean countries has been considered possible. The potential hazard for Cuba is therefore real. For this reason, a surveillance system in high-risk Cuban waters for *Vibrio cholerae* and methodology for its isolation were implemented on the basis of earlier studies done in various aquatic ecosystems during 7 yr by the water microbiology laboratory of National Institute of Hygiene, Epidemiology and Microbiology (INHEM).

The identification of the *Vibrio* species by this national monitoring system is described herein. Additional information on the distribution of other related species in different provinces of Cuba is given to elucidate the potential for the entry of related pathogens into the country.

Materials and methods.—During the years 1995, 1996, and the first 6 mo of 1997, samples containing strains of the families *Vibrionaceae* and *Aeromonadaceae* were collected and transferred from laboratories within the Cuban Provincial Centers of Hygiene, Epidemiology and Microbiology to INHEM for species identification. These water samples were analyzed by concentration methods (Moore swabs and Membrane Filtration) to identify *V. cholerae,* following the methodology of the American Public Health Association (1993) and the West and Colwell (1984) recommendations. Concentrated samples were cultivated in Alkaline Peptone Broth at 37 C and, after incubation for 6–8 and 18–20 hr, portions were streaked for isolation on TCBS agar and MacConkey agar, which were incubated for 18–24 hr at 37 C. After this time, and depending on the characteristics of the culture medium, three presumptive colonies were selected and seeded in Kligler iron agar (KIA) for later characterization with other biochemical tests, following the recommendations of West and Colwell (1984) and Alsina and Blanch (1994). Presumptive *Vibrio* isolates were cultivated for confirmation in Tryptone soy broth at 37 C for 6–8 hr. After this, an inoculum was seeded in TCBS Agar and Tryptone soy agar at 37 C for 18–24 hr. Three colonies were selected to complete purification and inoculation in KIA. Depending on these results, the biochemical and serological tests for identification and characterization were carried out. Strains identified as *V. cholerae* were tested with polyvalent antiserum *V. cholerae* O1. If there was no agglutination, they were classified as *V. cholerae* non-O1. The remaining strains of family *Vibrionaceae* were identified following the methods of West and Colwell (1984) and Alsina and Blanch (1994). Strains found to belong to the family *Aeromonadaceae* were classified by species according to the recommendations of Carnahan et al. (1991) and Furuwatari et al. (1994).

TABLE 1. Isolates identified as *Vibrio* and *Aeromonas* from Cuban waters.

Water sources	No. *Vibrio* strains (%)	No. *Aeromonas* strains (%)
Water supply	92 (16.4)	22 (10.0)
Wastewater	225 (45.4)	148 (65.0)
Coastal water	148 (26.2)	49 (21.0)
Rivers	67 (12.0)	9 (4.0)
Total	562 (100.0)	228 (100.0)

Results and discussion.—During the study period, 562 isolates of genus *Vibrio* were identified in water samples. The major percentage of these (45.4%) was in domestic wastewater, followed by coastal waters that receive surface drainage from terrestrial waters (26.2%), water supplies (16.4%), and rivers (12.0%) (Table 1). The high incidence of *V. cholerae* non-O1 in domestic wastewater suggests that gastrointestinal infections occur among the human population utilizing these facilities. These infections may be linked to the drinking water supply, because a significant percentage of the isolates were recovered from drinking water. Bacteria of this genus are autochthonous in the aquatic environment, mainly in brackish water with sufficient nutrients. Moreover, it has been established that the presence of *V. cholerae* is associated with plankton and other marine organisms. Table 1 shows that 228 isolates were identified as genus *Aeromonas,* with the major percentage (65.0%) found in wastewater. These species are typical in freshwater having a high content of nutrients. *Vibrio* species identified are shown in Table 2. The potential presence of *V. cholerae* O1 was a major concern of this study but fortunately was not detected. However, numerous strains of *V. cholerae* non-O1 were detected. A total of 471 isolates of *V. cholerae* non-O1 were identified. Other authors have reported on virulence factors in environmental strains of *V. cholerae* O1 and their potential importance for human health. *V. cholerae* non-O1 was thought to cause only sporadic cases of gastroenteritis, wound infections, and septicemia related to seafood consumption or exposure to the aquatic environment. Recently, the greater clinical importance of *V. cholerae* non-O1 has been shown because of the appearance of a new strain, *V. cholerae* serogroup O139, which was responsible for the cholera epidemic in India and Bangladesh (Morris, 1990).

V. mimicus was detected in several sources (wastewater, coastal water, and river) in 16 samples. This species is similar to *V. cholerae* in its ecology and biochemical behavior, except that it does not ferment sucrose. *V. mimicus* has been associated with gastroenteritis after seafood consumption, mainly raw oysters (Chowdhury et al., 1989). Regarding the halophilic species identified mostly in coastal waters, some have clinical importance: *V. alginolyticus* (29 isolates), *V. fluvialis* (15 isolates), *V. vulnificus* (6 isolates), *V. furnisii* (4 isolates), and *V. damsela* (1 isolate). In recent studies conducted in Florida and along the U.S. Gulf Coast, these species have been associated with illness from raw oyster consumption and infections acquired by water contact during recreation in marine waters (Kelly, 1991; Levine and Grifin, 1993; Hlady and Klontz, 1996).

Aeromonas species are showed in Table 3. The predominant species was *A. caviae* (124 strains) predominantly in wastewater, followed by *A. hydrophila* (87 isolates). These two species

TABLE 2. Number of *Vibrio* isolates from various environmental reservoirs.

Species	Water supply	Wastewater	Coastal water	Rivers	Total
V. cholerae, non-O1	89	226	94	62	471
V. alginolyticus	3	13	13	—	29
V. mimicus	—	6	6	4	16
V. fluvialis	—	4	11	—	15
V. pelagius, I	—	—	7	—	7
V. vulnificus	—	2	4	—	6
V. furnisii	—	—	4	—	4
V. anguillarum-like	—	—	4	—	4
V. damsela	—	—	1	—	1
V. logei	—	—	1	—	1
Halophilic *Vibrios* without identification	—	4	2	1	7
Total	92	255	147	67	561

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TABLE 3. Number of *Aeromonas* isolates from various environmental reservoirs.

Species	Water supply	Waste-water	Coastal water	Rivers	Total
A. caviae	10	87	25	2	124
A. hydrophila	12	47	23	5	87
A. veronii biovar *sobria*	—	7	—	1	8
A. trota	—	1	1	1	3
A. schubertii	—	2	—	—	2
P. shigelloides	—	4	—	—	4
Total	22	148	49	9	228

have been detected frequently in research elsewhere (Chowdhury et al., 1990; González et al., 1995), especially the anaerogenic species *A. caviae,* which is observed commonly in wastewater. *A. hydrophila,* could be enterotoxigenic; therefore, water containing these species presents human health risks.

The numbers of identified strains distributed among the provinces can be observed in Table 4. The principal locations were in Las Tunas, Cienfuegos, Holguin, and Habana provinces.

In conclusion, *V. cholerae* non-O1 was the species most frequently identified by this study. Because the habitat of these bacteria is thought to be similar to *V. cholerae* O1 and *V. cholerae* O139, there are favorable conditions for the survival and propagation of these pathogenic forms in the event of introduction into the Cuban aquatic environments.

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TABLE 4. Number of isolates for each bacterial species from various Cuban provinces.[a]

Species	CH	HA	CF	SS	VC	CA	LT	HO	GR	GU	SG	Total
V. cholerae non-O1	10	67	98	4	24	3	150	62	37	16	1	472
V. alginolyticus	14	2	—	—	—	—	5	2	—	6	—	29
V. mimicus	—	3	5	1	—	—	—	7	—	—	—	16
V. fluvialis	1	10	—	—	2	—	—	—	1	1	—	15
V. pelagius I	—	7	—	—	—	—	—	—	—	—	—	7
V. furnisii	—	4	—	—	—	—	—	—	—	—	—	4
V. anguillarum	—	4	—	—	—	—	—	—	—	—	—	4
V. damsela	—	1	—	—	—	—	—	—	—	—	—	1
V. harveyi	—	1	—	—	—	—	—	—	—	—	—	1
V. logei	—	1	—	—	—	—	—	—	—	—	—	1
Halophilic *Vibrios* without identification	—	2	1	—	—	—	1	—	2	1	—	7
A. caviae	11	15	4	6	51	—	18	2	11	6	—	124
A. hydropohila	2	13	14	8	10	—	16	2	5	16	—	86
A. veronii biovar *sobria*	—	—	1	—	—	1	4	—	1	1	—	8
A. trota	—	1	—	—	—	—	1	—	1	—	—	3
A. schubertii	2	—	—	—	—	—	—	—	—	—	—	2
P. shigelloides	2	—	—	—	—	—	—	—	2	—	—	4
Total												784

[a] Provinces: CH, Ciudad de la Habana; HA, Provincia Habana; CF, Cienfuegos; SS, Sancti Spiritus; VC, Villa Clara; CA, Camaguey; LT, Las Tunas; HO, Holguin; GR, Granma; GU, Guantánamo; SG, Santiago de Cuba.

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DROUGHT-INDUCED DECLINE OF SUBMERGED AQUATIC VEGETATION IN ESCAMBIA BAY, FLORIDA.—The submerged aquatic vegetation (SAV) in Upper Escambia Bay has been increasing in coverage over the past two decades, as was recently documented by Lores et al. (2000). *Vallisneria americana* Michaux was the dominant species of SAV in Upper Escambia Bay, and the area covered was >1.5 km^2. From 1950 to the 1970s, the SAV coverage in this area had declined. That decline was associated with increased nutrients and turbidity (Olinger et al., 1975). A recent dramatic decline apparently occurred over a period of a few months in early summer 2000.

Observations.—Locally, the recent decline of SAV was first noticed in Blackwater Bay, Florida, by N. Craft of the Northwest Florida Aquatic Preserves (pers. comm.). High salinity persisted throughout the summer and fall of 2000 because of a severe drought that has affected much of the southeast United States. Data from EPA's Gulf Ecology Division monthly monitoring of water quality parameters in profile at a station near the mouth of Escambia River (location indicated on Fig. 1) indicate that salinity was >12 at the surface from Oct. 1999 through Feb. 2000 and >15 at the surface during each of the monthly visits from June 2000 through Nov. 2000 (Table 1). This exceeds the *V. americana* salinity tolerance of 12 reported by Twilley and Barko (1990) and 15 reported by Kraemer et al. (1999). As a result, most of the area that was previously covered by SAV beds (Lores et al., 2000) is now bare sand/mud bottom.

Monitoring in July 2000 confirmed the decline in Escambia Bay. Observation suggested that *V. americana* was suffering adverse impacts of high salinity. Large rafts of *V. americana* blades were found in sheltered coves, and piles of fresh wrack were visible along the shore. Whole floating plants with no attached roots were also observed, one of the signs of salinity stress noted by Kraemer et al. (1999). Some remnant beds were observed but not mapped during that visit. In September 2000, we conducted a new mapping with a High-Precision Global Positioning System, A CMT (Corvallis MicroTechnology) model HP-GPS-L4, which uses a 12-channel Leica receiver, yielding a nominal postprocessed accuracy of 60 cm.

We documented the position of remaining beds and the absence of SAV along much of the northwestern corner of Escambia Bay, where the most dense beds had existed in 1998 (Fig. 1). The remaining *V. americana* grass beds were <0.3 m tall and frequently <0.1 m tall. This is in contrast to previous years, at this time when *V. americana* was generally taller than 0.5 m and flowering profusely. No evidence of reproduction was observed in any of the remaining beds. The remnant beds that were found were in very shallow water (<0.5 m). Also notable during this visit was the condition of emergent marsh vegetation, much of which had receded and appeared to be stunted, dormant, or dead. Subsequent to the Sep. mapping, and because of the continuation of

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